Genotyping of NDRG1-floxed mice

1. The original method provided by the original depositors

This chapter describes the genotyping method the original depositors provided. Three primers are used for genotyping of NDRG1 floxed mice.

1.1) Primer sequences:

- Primer1: 5'-CCG CCT CTG TCA AAT TAG TAG CTG-3' (24-mer)
- Primer2: 5'-GGG AGA GCT GAA GGC TGT TCT AGG-3' (24-mer)
- Primer3: 5'-ACA GCC TCG ATC GAG GAA TTC C-3' (22-mer)

1.2) Reaction mixture:

	μL
Water	3
Primer1 (10 μM)	2
Primer2 (10 μM)	2
Primer3 (10 μM)	2
HotStarTaq Master Mix	10
DNA from a ear-punched tissue (one piece / 200μL)	1
total	20

Taq polymerase: HotStarTaq Master Mix Kit (Qiagen). The enzyme is a chemically modified Taq polymerase for hot start PCR and needs 15-min incubation at 95 °C for activation. Master Mix contains enzyme, dNTP, Mg, etc. at 2 x concentration. Please see Qiagen's website for details (http://www1.qiagen.com/Products/Pcr/HotStarTaqSystem/HotStarTaqMasterMix.aspx).

1.3) Thermal cycles:

95 °C	15 min	once
94 °C	15 sec	35 cycles
60 °C	15 sec	
72 °C	20 sec	
72 °C	3 min	once

No information about thermal cyclers was provided by the original depositor.

1.4) Product size:

Primers 1 and 2: approx. 200 bp for wild-type alleles
Primers 2 and 3: approx. 240 bp for floxed alleles

Reference

Okuda T, Higashi Y, Kokame K, Tanaka C, Kondoh H, Miyata T. Ndrg1-deficient mice exhibit a progressive demyelinating disorder of peripheral nerves. Mol Cell Biol. 2004, 24(9):3949-56. (PMID: 15082788)